REMARKS

I. STATUS OF CLAIMS

Claims 1-4, and 7 are pending in this application. Claims 5-6 and 8-65 are canceled. Pending claims 1-4, and 7, as set forth in the Claims listing, are rejected.

II. SUMMARY OF CLAIMED SUBJECT MATTER

Claims 1 and 7 are independent claims and claims 2-4 are dependent on claim 1. Each of the independent claims 1 and 7 are directed to highly purified preparations of glycosylated polypeptides comprising a CD44 amino acid backbone and sialylated, fucosylated glycans and having E-selectin or L-selectin ligand activity.

CD44 is known to function as an adhesion molecule. Adhesion molecules are responsible for maintaining the specialized cytoarchitecture of the hematopoietic microenvironment that is created by discrete cell-cell and cell-matrix adhesive interactions that are tightly regulated by cell lineage-specific expression of adhesion molecules.[1/] CD44 is a broadly distributed cell surface glycoprotein receptor expressed on a diverse variety of cell types including most hematopoietic cells, keratinocytes, chondrocytes, many epithelial cell types, and some endothelial and neural cells. CD44 is known to participate in a wide variety of cellular functions, including cell-cell aggregation, retention of pericellular matrix, matrix-cell and cell-matrix signaling, receptor-mediated internalization/degradation of hyaluronan, and cell migration.[2/] CD44 is also referred to as "hyaluronic acid receptor" because its cellular function was initially thought to be dependent on CD44-hyaluronan interactions.[3/]

There are several CD44 isoforms that each differ, *inter alia*, in primary amino acid sequence and/or cell or tissue expression patterns. Such isofroms include as CD44H, CD44E, CD44R1, and CD44R2, to name a few. Independent claim 1 recites a particular isoform of

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1/ See Specification at paragraph [0004].

2/ See Specification at page 9, lines 7-14.

3/ See Specification at page 9, lines 7-14.

CD44 (*i.e.*, CD44H), which is used to describe the amino acid backbone for the recited glycosylated CD44 polypeptides. Specifically, claim 1 recites a purified preparation of a glycosylated CD44 polypeptide that comprises an amino acid backbone encoded by a nucleotide sequence comprising exons 1-5, 16, 17, 18, and 20 of a human CD44 gene.[4/]

Claim 1 is directed to purified preparation comprising particular glycoforms of the above-described CD44 isoform. Specifically, claim 1 recites that the CD44 polypeptide is glycosylated with sialylated, fucosylated glycans. The particular glycosylation patterns are generally cell or tissue dependent and are the result of the expression of glycosyltransferase enzymes such as FucTIV, FucTVII and of ST3Gal IV that are involved in the glycosylation of polypeptides and which catalyze the transfer of particular carbohydrates to the polypeptide backbone.[5/] Such physiologic modifications of the CD44 polypeptide backbone control the binding characteristics and thus the biological activity of the glycoforms.[6/] In the present case, the glycosylated CD44 polypeptides of the claims possess E-Selectin and/or L-Selectin binding activities. Further, these particular glycosylation patterns also affect the binding of the HECA-452 monoclonal antibody. The present invention clearly demonstrated that the recited glycosylated CD44 polypeptides comprising distinguishing sialylated, fucosylated glycans possess a novel reactivity with HECA-452 monoclonal antibody.[7/] Examples 2 and 3 shows that HECA-452 reactivity was associated with E-selectin ligand activity, and Example 6 shows that the HECA-452 reactivity was associated with L-selectin activity. Taken together, the specification provides evidence that the glycosylated CD44 polypeptides of the present claims are reactive with monoclonal antibody HECA-452, which recognizes sialofucosylated glycans. The presence of these sialofucosylated glycans confers L-selectin and E-selectin binding.

The specification provides that the glycosylated CD44 polypeptides of the claims can be evaluated for one or more of the functional activities, *i.e.*, E-selectin and/or L-selection binding activity. E-selectin activity may be evaluated using for example the methods and assays

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4/ See *e.g.*, Specification at page 9, lines 15-30.

5/ See *e.g.*, Example 12 of the Specification beginning of page 64.

6/ See e.g., Example 8 of the Specification beginning on page 48.

7/ See e.g., Figure 1A and Example 1 beginning on page 37.

described in Examples 3 and 4 of the Specification.[8/] L-selectin activity may be evaluated using for example the methods and assays described in Example 8 of the Specification.[9/]

Claim 1 further recites that the preparation comprises less than 5% of a polypeptide other than the glycosylated CD44 polypeptide. Purified preparations containing less than 5% of a polypeptide other than the CD44 glycoform of the present invention is described on page 11, lines 13 to 22.

Independent claim 7 recites a purified preparation of a glycosylated polypeptide comprising the amino acid sequences of SEQ ID NO: 1 and sialylated, fucosylated glycans. The claimed purified preparation of claim 7 primarily differs from claim 1 in that the polypeptide backbone comprises the amino acid sequence of SEQ ID NO: 1. SEQ ID NO: 1 references the amino acid sequence of a CD44 isoform described as CD44R1 (Gen Bank Acc. CAA40133), which was originally described in Dougherty.[10/]

III. ARGUMENT

A. The Teachings of Sackstein 1997 Fail to Describe Each And Every Claim Element and Therefore Cannot Anticipate Claims 1-4 and 7

Claims 1-4 and 7 have been rejected under 35 U.S.C. § 102(b) as being unpatentable over Sackstein 1997, as further evidenced by Dimitroff and Sackstein 2004 Review.

In order for Sackstein to qualify as an anticipatory reference, the reference must described each and every element of the claim, either expressly or inherently.[11/] Sackstein 1997, however, fails to describe each and every limitation of claims 1-4 and 7. In the least, Sackstein

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8/ Beginning on page 41 of the Specification.

9/ Beginning on page 48 of the Specification.

10/ Dougherty GJ, Landorp PM, Cooper DL, Humphries RK.; "Molecular cloning of CD44R1 and CD44R2, two novel isoforms of the human CD44 lymphocyte 'homing' receptor expressed by hemopoietic cells;" J Exp Med. 1991 Jul 1;174(1):1-5.

11/ *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987)("A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference.").

1997 fails to disclose purified preparations of a glycosylated polypeptide comprising a CD44 amino acid backbone and sialylated, fucosylated glycans.

Sackstein 1997 identified the existence of an unknown, non-descript molecule having L-selectin binding activity that is expressed in the human hematopoietic cell line KG1a. Thus, Sackstein 1997 identifies an activity by way of a functional assay — that is, a glycoprotein had been identified by its activity, however, the actual identity and subsequent purification of the glycoprotein was yet to be determined.[12/] With respect to the structure, Sackstein 1997 merely disclosed that this unidentified glycoprotein was not recognized by the MECA 79 antibody (an antibody to known L-selectin ligands)[13/] and, in contrast to other known L-selectin ligands, possesses binding activity that is not sulfate dependent.[14/] Sackstein describes a polypeptide that was not MECA-79 reactive and therefore could **not** be immunoprecipitated from KG1a lysates using a MECA-79 antibody. Accordingly, no purified preparation of the unidentified glycoprotein is disclosed in Sackstein 1997.

Sackstein 1997 therefore fails to disclose a preparation of a glycosylated CD44 polypeptide comprising, *inter alia*, sialylated, fucosylated glycans having the recited level of purity. Sackstein 1997, however, merely discloses the identification of an activity of an unknown protein contained with the whole cell lysates of KG1a cells. Sackstein 1997 fails to describe the successful purification of the protein having the identified activity.

The Final Office Action argues, however, that Sackstein 1997 discloses "efforts to isolating and characterizing the structure of the KG1a ligand", or that "one of ordinary skill in the are would have immediately envisaged isolated HCELL/KG1a CD44 protein."[15/] These conclusions, however, are not factually support. Nowhere does Sackstein 1997 describe the successful purification of the protein having the identified activity. Further, *nowhere* does Sackstein 1997 even mention CD44 as a possible candidate for unknown, non-descript

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12/ See *e.g.*, Sackstein 1997 at Abstract.

13/ See *e.g.*, Sackstein 1997 at page 2773, left column, second full paragraph for general discussion related to the MECA 79. For results of experiments using MECA 79, see Figure 1 and 2 and related discussion indicating that the KG1a cell does not contain MECA 79 determinants.

14/ See *e.g.*, Sackstein 1997 at page 2780, left column, first full paragraph.

15/ Office Action mailed 5/21/2008 at page 5, lines 1-5.

glycoprotein having L-selectin binding activity discussed therein. At best, Sackstein 1997 shows immunoprecipitation experiments in which whole cell KG1a lysates were placed on a gel, but no protein(s) relevant to the sialofucosylated CD44 are isolated.

Further, the protein gels discussed in Sackstein 1997 are relied on by the Examiner are not purified preparations "containing less than 5% of a polypeptide other than the glycosylated CD44 polypeptide." Whole cell KG1a lysates were loaded onto the gels and these lysates would contain most, if not all, proteins from the KG1a cells. This is not a purified preparation containing less than 5% of any other polypeptide. Accordingly, Sackstein 1997 cannot anticipate the present claims because this reference, in the least, does not disclose a purified preparation of a glycosylated CD44 polypeptide having sialylated, fucosylated glycans, wherein the preparation comprises less than 5% of a polypeptide other than the glycosylated CD44 polypeptide, as is recited in the claims. The Office Action is setting forth a rationale that is inconsistent with the standard of anticipation under 35 U.S.C. § 102.

Nothing disclosed in the other references cited by the Examiner as extrinsic evidence of anticipation (Dimitroff and Sackstein 2004 Review) changes these facts. The Office Action relies on Dimitroff and Sackstein 2004 Review, the present inventors own work, as evidence that the unknown, non-descript glycoprotein having L-selectin binding activity described in Sackstein 1997 is the CD44 glycoform of the pending claims. Even assuming, *arguendo*, that the polypeptide of Sackstein is the same as the recited polypeptide, the Dimitroff and Sackstein 2004 references at best merely document the difficulty in identifying the CD44 glycoform of the present claims. Neither Dimitroff and Sackstein 2004 qualify as prior art.

Applicant requests that this rejection be reversed.

B. The Teachings of Stamenkovic Fail to Describe Each And Every Claim Element and Therefore Cannot Anticipate Claims 1-4 and 7

Claim 1-4 and 7 have been rejected under 35 U.S.C. § 102(b) as being unpatentable over Stamenkovic, as further evidenced by Sackstein 2003 and Sackstein 2004 Review.

Stamenkovic, however, is not an anticipatory reference. Stamenkovic merely teaches that there are two isoforms of CD44: an epithelial form of the CD44 polypeptide, which is distinct

from the hematopoietic/mesodermal form.[16/] Specifically, Stamenkovic describes that the epithelial form contains an additional extracellular peptide domain interposed proximal to the membrane-spanning domain and that this additional peptide sequence impairs binding to the extracellular matrix element hyaluronate. Stamenkovic demonstrates these two forms of CD44 by analysis of CD44 transcripts (*i.e.* RNA) from various cell types.[17/]

In order for Stamenkovic to qualify as an anticipatory reference, however, the reference must describe each and every element of the claim, either expressly or inherently.[18/] Stamenkovic fails in this regard. Stamenkovic fails to disclose a CD44 glycoform comprising any sialylated, fucosylated glycans. Stamenkovic fails to disclose a CD44 glycoform having L-selectin binding activity. Stamenkovic fails to disclose sterile or purified preparations according to the claims for any protein.

At best, the rejection set forth on the Office Action is based on the principles of inherency in that the polypeptide of Stamenkovic is inherently the same as the recited polypeptide. Anticipation by inherency, however, requires that the prior art reference disclose each and every limitation of the claim.[19] Nowhere in Stamenkovic is a disclosure of a preparation of a glycosylated CD44 polypeptide comprising, *inter alia*, sialylated, fucosylated glycans having the recited level of purity. In order for a prior art reference to anticipate a claim under the principles of inherency, the prior art reference must ***necessarily*** function in accordance with, or include, all claimed limitations in order to anticipate the claim.[20] A conclusion that a result or characteristic ***may be present in the prior art reference is insufficient***, as provided by M.P.E.P. § 2112 as follows (citations omitted):

> The fact that a certain result or characteristic may occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic. "To establish inherency, the extrinsic evidence 'must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so

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16/ *See* Stamenkovic at page 346, first full paragraph.

17/ *See* Stamenkovic at Figure 2.

18/ *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987)("A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference.").

19 *See Standard Havens Prods., v Gencor Indus., Inc.*, 953 F.2d 1360, 1369 (Fed. Cir. 1991).

20 *See In re King,* 801 F.2d 1324, 1326 (Fed. Cir. 1986).

> recognized by persons of ordinary skill. Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient.'".

Additionally, as stated in *Glaverbel Societe Anonyme v. Northlake Marketing & Supply Inc.*, 45 F. 3d, 1550 (Fed. Cir. 1995)(citations omitted):

> Anticipation, however, requires identity of invention; the claimed invention, as described in appropriately construed claims, ***must be the same as that of reference***, in order to anticipate.

Applicant respectfully submits that Stamenkovic fails to inherently disclose a polypeptide that is ***necessarily*** a glycosylated CD44H polypeptide comprising sialylated, fucosylated glycans. Indeed, Figure 3 of Stamenkovic demonstrates immunoprecipitation of the "hematopoietic form" of CD44 (CD44H) from CD44H transfected COS cells. [Figure 4 of Stamenkovic demonstrates the immunprecipitation of CD44 from carcinoma cell lines (*i.e.*, the epithelial form) not the hematopoietic form.] The COS cell line is derived from kidney cells of the African Green monkey. As is explained in a Declaration by Dr. Sackstein filed July 7, 2007, CD44H-transfected COS cells cannot produce the claimed glycosylated polypeptide as COS cells are known to lack relevant fucosyltransferases (particularly fucosyltransferase VII), which are essential for producing the sialofucosylated selectin binding determinants of the claimed glycosylated polypeptide.[21/] CD44H-transfected COS cells thus cannot produce the claimed glycosylated polypeptide as COS cells natively lack the relevant fucosysltransferase to create such modifications.

Further, the Office Action at page 7, second full paragraph argues that Stamenkovic teaches the "isolation of hemopoietic and epithelial forms of CD44…, including that the hemopoietic form shows a species of molecular mass of approximately 80kD." In this passage, the Examiner is referring to Figure 3 on page 345 of Stamenkovic and related discussion. Stamenkovic teaches that Figure 3 also shows the epithelial form of the CD44 polypeptide (CD44E) at 130 kD and the hematopoietic/mesodermal form (CD44H) at 80 kD. Compare this to what is described in the specification at Example 4: *Identification and Characterization of HCELL*,[22/] which teaches that the major L-selectin ligand activity can be found at 98 kD species.

21/ Declaration under 37 C.F.R. §1.132 by Dr. Sackstein filed July 10, 2007.

22/ See page 42 of the Specification.

Stamenkovic does not disclose this species. Indeed, a close examination of Figure 4 of Stamenkovic clearly shows that the molecular weight of the immunoprecipitated species is 80 kD, or in the least, well under 92 kD. One explanation for this difference is that Stamenkovic is describing the form of CD44 that is known to bind to HA, and which is smaller in size because lacks certain modifications with complex carbohydrates. Further, CD44R1, or SEQ ID NO: 1, is even larger since the amino acid backbone contains an additional 132 amino acids.[23/] Accordingly, Stamenkovic is not relevant to claim 7.

More importantly, immunoprecipitation of CD44 from the cells as described in Stamenkovic could not produce a preparation of comprising less than 5% of a polypeptide other than the recited glycosylated CD44 polypeptide. In order to achieve this recited level of purity, a person of ordinary skill in the art would had to at least recognize the sialylated, fucosylated glycans as structural components of CD44 and then first immunoprecipitate CD44 and then immunoprecipitate with an antibody that recognizes the distinguishing glycans (e.g., HECA-452 or a E-selectin-immunoglobulin chimera). However, one would had to have known that a specialized sialylated, fucosylated glycoform of CD44 exists that is an E-selectin ligand. The references cited by the Examiner provide no such knowledge or suggestion. Accordingly, Stamenkovic provides no guidance as to how to achieve the recited level of purity for the recited glycoform of CD44.

In view of the above, Applicants respectfully submit that the Examiner is apply an improper standard for assessing anticipation. Further, it cannot be said that Stamenkovic necessarily discloses the CD44 glycoforms recited in the present claims. Applicant requests that this rejection be reversed.

C. The Teachings of Dougherty Fail to Describe Each And Every Claim Element and Therefore Cannot Anticipate Claims 1-4 and 7

Claims 1-4 and 7 have been rejected under 35 U.S.C. § 102(b) as being unpatentable over Dougherty. Dougherty discloses the amino acid sequences for the CD44 polypeptide backbones CD44R1 and CD44R2.

23/ See *e.g.*, Dougherty at page 2, right column.

Primarily, the Western blot protein gels discussed in Dougherty relied on by the Examiner are not purified preparations "containing less than 5% of a polypeptide other than the glycosylated CD44 polypeptide." Whole cell KG1a lysates were loaded onto the gels and these lysates would contain most, if not all, proteins from the KG1a cells. This is not a purified preparation containing less than 5% of any other polypeptide. CD44 itself was not purified, nor was any distinct glycoform of CD44 purified. Accordingly, Dougherty cannot anticipate the present claims because this reference, in the least, does not disclose a purified preparation of a glycosylated CD44 polypeptide having sialylated, fucosylated glycans, wherein the preparation comprises less than 5% of a polypeptide other than the glycosylated CD44 polypeptide, as is recited in the claims. The Office Action is setting forth a rationale that is inconsistent with the standard of anticipation under 35 U.S.C. § 102. Accordingly, Dougherty can not anticipate the present claims.

More importantly, immunoprecipitation of CD44 from the cells as described in Dougherty could not produce a preparation of comprising less than 5% of a polypeptide other than the recited glycosylated CD44 polypeptide. In order to achieve this recited level of purity, a person of ordinary skill in the art would had to at least recognize the sialylated, fucosylated glycans as structural components of CD44 and then first immunoprecipitate CD44 and then immunoprecipitate with an antibody that recognizes the distinguishing glycans (e.g., HECA-452 or a E-selectin-immunoglobulin chimera). However, one would had to have known that a specialized sialylated, fucosylated glycoform of CD44 exists that is an E-selectin ligand. The references cited by the Examiner provide no such knowledge or suggestion.

Accordingly, Dougherty provides no guidance as to how to achieve the recited level of purity for the recited glycoform of CD44.

Nonetheless, the Examiner on page 10 of the Final Office Action maintains the following:

> Again it is maintain that given the teachings including the Discussion of efforts to isolating and characterizing the structure of the KG1a ligand by Sackstein *et al.* at the time of the invention, one of ordinary skill in the art would have immediately envisaged isolated HCELL / KG1 a CD44 protein, including 'isolated or purified protein that is substantially free of cellular materials or other contaminating protein from the cell or tissue source form which HCELL glycoprotein is derived or substantially freed form chemical precursors or other chemical when chemically synthesized , including being

> recombinantly produced, which is also free of culture medium (e.g. see page 11, paragraph 3 of the instant specification), thereby meeting the claimed limitation of "wherein the preparation comprises less that 5% of a polypeptide other than the glycosylated CD44 polypeptide'.

It is clear from a reading of Dougherty and from the above reliance of Dougherty in the Final Office Action that Dougherty does not teach the purified preparation of the present claims. The Examiner is setting forth a rationale that is inconsistent with the standard of anticipation under 35 U.S.C. § 102 by maintaining that "one of ordinary skill in the art would have been immediately envisaged" the purified preparation having "less than 5% of a polypeptide other than the glycosylated CD44 polypeptide." In the least, Dougherty fails to teach this element and therefore cannot anticipate the present claims

Furthermore, Dougherty fails to identify any glycoforms of CD44. As indicated by the specification, there are numerous CD44 isoforms and glycoforms with distinct functional activities and the Examiner presents no convincing evidence that the polypeptides of Dougherty are the same glycoproteins recited in the claims. At best, the rejection set forth in the Office Action is based on the principles of inherency in that the polypeptide of Dougherty inherently discloses glycosylated polypeptides comprising sialylated, fucosylated glycans and having E-selectin ligand activity, L-selectin activity, or both. Anticipation by inherency, however, requires that the prior art reference disclose each and every limitation of the claim.[24/] As explained in the present Specification, the glycosylation patterns of CD44 glycoform affects binding activity of CD44 isoforms, which include the ability to bind to certain monoclonal antibodies. The present invention clearly demonstrates that the recited glycosylated CD44 polypeptides comprising sialylated, fucosylated glycans possess a novel reactivity with HECA-452 monoclonal antibody and possesses E-selectin and/or L-selectin binding activity.[25/] Dougherty, however, identified CD44R1 with Western blots using the monoclonal antibodies 8d8 and 50B4.[26/] By way of example, Sackstein 1997 unsuccessfully attempted to identify a protein having L-selectin activity with the MECA 79 antibody (an antibody to known bind to L-

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24/ See *e.g., Standard Havens Prods., v Gencor Indus., Inc.*, 953 F.2d 1360, 1369 (Fed. Cir. 1991).

25/ See *e.g.*, Specification at Figure 1A and Example 1 beginning on page 37.

26/ See Dougherty at Page 3, Figure 1 legend.

selectin ligands).[27/] Because glycoproteins can display distinct binding activities even where the amino acid backbone is the same or similar, it cannot be said that Dougherty necessarily discloses the glycoproteins of the present claims.

Furthermore, Dougherty does not disclose particular CD44 glycoforms. The contribution to the art in Dougherty was the identification of the amino acid sequences for the CD44 isoforms CD44R1 and CD44R2. At best, Dougherty identifies the primary sequence of CD44R1, and any characterization of CD44R1 presented in Dougherty is merely based off of the analysis of that primary sequence. Glycoforms of CD44R1 are not disclosed in Dougherty.

In view of the above, Dougherty cannot anticipate the present claims. Applicant requests that this rejection be reversed.

D. Rejections On Obviousness Are Sustained By Mere Conclusory Statements And Not A Clear, Articulated Rationale

Claims 1-4 and 7 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Sackstein 1997, Stamenkovic, and/or Dougherty (explained above), in view of Ni *et al.* (U.S. Patent No. 5,942,417; "Ni") and McEver *et al.* (U.S. Patent No. 6,124,267; "McEver"). Applicants disagree with this rejection for the reasons below.

The section headings used herein are for organizational purposes only; arguments used under the heading of one reference may equally apply across all references.

1. A CD44 Glycoform Comprising Sialylated, Fucosylated Glycans is Not Taught in Any Reference Relied On By The Examiner

As presented above, Sackstein 1997, Stamenkovic, and Dougherty each fail to expressly or inherently disclose the recited purified preparation of CD44 glycoform accordingly to the present claims. In maintaining this rejection, the Examiner has failed to appreciate the distinction between CD44 isoforms, which concerns the amino acid backbone, and the currently claimed glycoforms of CD44, which concerns the post-translational modification of the polypeptide

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27/ See *e.g.*, Sackstein 1997 at page 2773, left column, second full paragraph for general discussion related to the MECA 79. For results of experiments using MECA 79, see Figure 1 and 2 and related discussion indicating that the KG1a cell does not contain MECA 79 determinants.

backbone with carbohydrates. Indeed, each of the above references never disclose a CD44 glycoform comprising sialylated, fucosylated glycans having the recited activities.

a. Sackstein 1997 does not disclose any CD44 glycoform

Sackstein 1997 discloses the identification of an activity of an unknown protein contained within the whole cell lysates of KG1a cells. Sackstein 1997 fails to describe the successful purification of the protein having the identified L-selectin binding activity. Sackstein 1997 fails to identify the polypeptide as having a CD44 amino acid backbone. Sackstein 1997 fails to discloses any CD44 glycoform, and specifically, a CD44 glycoform comprising sialylated, fucosylated glycans an having the recited activities.

In the least, the Examiner fails to provide any rationale as to how Sackstein 1997 would have led a person of ordinary skill to a CD44 glycoform comprising and sialylated, fucosylated glycans, and fails to provide any rationale for its purification. Indeed, Sackstein 1997 teaches away from CD44 as a possible candidate for the molecule displaying L-selectin activity. Sackstein 1997, in the Abstract, specifically states that the "native membrane L-selectin ligand exhibit[s] sulfate-independent function." Maiti[28/], published in 1998 in the reputable journal *Science*, is offered as evidence that a person of ordinary skill in the art would have consider that sulfation was required for CD44-mediated leukocyte adhesion. Specifically, Maiti expressly teaches as follows:

> The proinflammatory cytokine tumor necrosis factor-α, but not interferon-γ, was found to convert CD44 from its inactive, nonbinding form to its active form by inducing the sulfation of CD44. This posttranslational modification was required for CD44-mediated binding to the extracellular matrix component hyaluronan and to vascular endothelial cells.[29/]

In contrast, Sackstein 1997 expressly teaches that the identified L-selectin binding activity was sulfate independent.[30/] That is, the molecule described in Sackstein 1997 possessed the ability to bind to L-selectin, a leukocyte glycoprotein that mediates adhesive interactions, in a reaction that did not require sulfation. As CD44 was thought to require sulfation, a person of

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28/ Maiti *et al.*, "TNF-alpha induction of CD44-mediated leukocyte adhesion by sulfation," Science. 1998 Oct 30;282(5390):941-3.

29/ Matai at Abstract.

30/ Sackstein 1997 at Abstract.

ordinary skill in the art would have been led to believe that the molecule described by Sackstein 1997 was not CD44.

The Examiner points to a post-filing publication, Sackstein 2004 Review, as evidence that the protein of Sackstein 1997 is not novel. Sackstein 2004 Review, however, is a review article, and with the benefit of hindsight, acknowledges that the molecule identified in Sackstein 1997 was "not novel per se", meaning that while the CD44 glycoform was novel, the CD44 amino acid sequence was previously described. Sackstein 2004 Review is a review of the present inventor's own work and simply chronologizes the inventive process. Importantly, the Abstract of the Sackstein 2004 Review clearly states that "Hematopoietic stem cells (HSC) express a novel glycoform of CD44 known as hematopoietic cell E-/L-selectin ligand (HCELL). This molecule is the most potent E-selectin ligand natively expressed on any human cell." The Abstract has more weight as it represents the synopsis of the entire document. It clearly states "a novel glycoform of CD44". The Examiner has lifted a single phrase from the body of the review and has misinterpreted its meaning: that phrase specifically makes reference to protein mass spectrometry data which identified CD44 as the polypeptide backbone. Thus, while CD44 itself was known, as such it is not novel, the GLYCOPROTEIN is still a NOVEL glycoform of CD44. There is no contradiction here.

As stated above, Sackstein 1997 identified the existence of an unknown, non-descript molocule having L-selectin binding activity that is expressed in the human hematopoietic cell line KG1a. Sackstein 1997 dose not suggest to a person of ordinary skill in the art would that the unknown molecule was CD44, let alone a CD44 glycoprotein with a CD44 amino acid backbone.

b. Stamenkovic does not disclose any CD44 glycoform

Stamenkovic is cited by the Examiner for its teaching of CD44H. Stamenkovic, however, does not disclose a purified preparation as defined by the claims comprising a CD44 glycoform comprising sialylated, fucosylated glycans and having E-selectin and/or L-selectin binding activity. In the least, the Examiner fails to provide any rationale as to how Stamenkovic would have led a person of ordinary skill to a CD44 glycoform comprising sialylated, fucosylated glycans, and fails to provide any rationale for its purification. A full reading of Stamenkovic establishes that the authors did not identify specific glycoforms of CD44, did not distinguish functional differences between CD44 glycoforms, and accordingly, could not lead

one of ordinary skill in the art to a purified preparation containing CD44 glycoforms comprising sialylated, fucosylated glycans. The main contribution of Stamenkovic is the identification of two CD44 isoforms by their amino acid sequence. Stamenkovic does not teach of suggest any specific CD44 glycoforms and does not articulate any functional relevance of such glycoforms.

Importantly, Stamenkovic further teaches that the hematopoietic form of CD44, or CD44H, recognizes hyaluronate (HA) and that "this recognition appears to account for all the previously proposed adhesion specificities."[31/] This description teaches away from a CD44H modified with carbohydrates, such as sialylated, fucosylated glycans, and possessing certain adhesion specificities such as the ability to bind E-selectin or L-selectin. Indeed, it was commonly believed in the art that complex carbohydrates on CD44 actually inhibited the ability of CD44 to mediate adhesive interactions. That is, CD44 is expressed on a variety of cell types but only a few appeared to use CD44 to mediate adhesive interactions. This variability was apparently due to the variability of CD44 to bind to HA, which according to Stamenkovic, "account[ed] for all the previously proposed adhesion specificities."[32/] It was also commonly known in the art that complex carbohydrates bound to CD44 actually inhibited the ability of CD44 to bind to HA. Katoh,[33/] for example, teaches that carbohydrate modifications, such as sialic acid, actually inhibit the ability of CD44 to bind to HA. Accordingly, a person of ordinary skill in the art would have expected that glycoforms of CD44 could not mediate adhesive interactions, because CD44 recognizes hyaluronate (HA) and it is "this recognition [that] appears to account for all the previously proposed adhesion specificities."[34/] Accordingly, a person of ordinary skill in the art could not have relied on the teachings of Stamenkovic to arrive at a CD44H molecule modified with sialic acid, let alone with sialylated, fucosylated glycans that serves as an adhesion molecule.

Furthermore, Stamenkovic cannot be relied up to teach a CD44H molecule that is fucosylated. Indeed, Figure 3 of Stamenkovic demonstrates immunoprecipitation of the "hematopoietic form" of CD44 (CD44H) from CD44H transfected COS cells. [Figure 4 of

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31/ Stamenkovic at page 343, right column, lines 3-7.

32/ Stamenkovic at page 343, right column, lines 3-7.

33/ Katoh S, Zheng Z, Oritani K, Shimozato T, Kincade PW.; "Glycosylation of CD44 negatively regulates its recognition of hyaluronan;" J Exp Med. 1995 Aug 1;182(2):419-29.

34/ Stamenkovic at page 343, right column, lines 3-7.

Stamenkovic demonstrates the immunprecipitation of CD44 from carcinoma cell lines (*i.e.*, the epithelial form) not the hematopoietic form.] The COS cell line is derived from kidney cells of the African Green monkey. As is explained in a Declaration by Dr. Sackstein dated July 7, 2007, CD44H-transfected COS cells cannot produce the claimed glycosylated polypeptide as COS cells are known to lack relevant fucosyltransferases (particularly fucosyltransferase VII), which are essential for producing the sialofucosylated selectin binding determinants of the claimed glycosylated polypeptide.[35/] CD44H-transfected COS cells thus cannot produce the claimed glycosylated polypeptide as COS cells natively lack the relevant fucosysltransferase to create HCELL.

Further, the Office Action at page 7, second full paragraph argues that Stamenkovic teaches the "isolation of hemopoietic and epithelial forms of CD44…, including that the hemopoietic form shows a species of molecular mass of approximately 80kD." In this passage, the Examiner is referring to Figure 3 on page 345 of Stamenkovic and related discussion. Stamenkovic teaches that Figure 3 also shows the epithelial form of the CD44 polypeptide (CD44E) at 130 kD and the hematopoietic/mesodermal form (CD44H) at 80 kD. Compare this to what is described in the specification at Example 4: *Identification and Characterization of HCELL*,[36/] which teaches that the major L-selectin ligand activity can be found at 98 kD species. Stamenkovic does not disclose this species. Indeed, Figure 4 of Stamenkovic clearly shows that the molecular weight of the immunoprecipitated species is 80 kD, or in the least, well under 92 kD. Further, CD44R1, or SEQ ID NO: 1, is even larger since the amino acid backbone contains an additional 132 amino acids.[37/] Accordingly, it can not be said that Stamenkovic necessarily discloses the CD44 glycoforms recited in the present claims.

c. Dougherty dose not disclose any CD44 glycoform

Dougherty is cited by the Examiner for its teaching of CD44R1 and CD44H. Dougherty, however, does not disclose a purified preparation as defined by the claims comprising a CD44 glycoform comprising sialylated, fucosylated glycans and having E-selectin and/or L-selectin

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35/ Declaration under 37 C.F.R. §1.132 by Dr. Sackstein filed July 10, 2007

36/ See page 42 of the Specification.

37/ See *e.g.*, Dougherty at page 2, right column.

binding activity. In the least, the Examiner fails to provide any rationale as to how Dougherty would have led a person of ordinary skill to a CD44 glycoform comprising sialylated, fucosylated glycans, and fails to provide any rationale for its purification. A full reading of Dougherty establishes that the authors did not identify specific glycoforms of CD44, did not distinguish functional differences between CD44 glycoforms, and accordingly, could not lead one of ordinary skill in the art to a purified preparation containing CD44 glycoforms comprising sialylated, fucosylated glycans. The main contribution of Dougherty is the identification of two CD44 isoforms (CD44R1 and CD44R2) by their amino acid sequence. Dougherty does not teach of suggest any specific CD44 glycoforms and does not articulate any functional relevance of such glycoforms.

d. The primary references cannot be applied equally to all claims.

Further, the references cannot be applied equally against claims 1 and 7. Independent claim 1 recites a purified preparation comprising a glycosylated CD44 polypeptide, wherein the glycosylated CD44 polypeptide is CD44H. Independent claim 7 recites a purified preparation of a glycosylated polypeptide, wherein the a glycosylated CD44 polypeptide comprises the amino acid sequences of SEQ ID NO: 1. Sackstein 1997 does not disclose any CD44 glycoform. Stamenkovic does not disclose CD44R1. Accordingly, these references are not cannot be relied upon to render obvious claim 7.

e. The secondary references, Ni and McEver, do not disclose any purified CD44 glycoform

Neither Ni nor McEver disclose a purified preparation according to the claims comprising any CD44 glycoform. Ni concerns the disclosure of a novel CD44-like protein receptor, and methods using and making thereof. The background section of Ni provides a summary of CD44 that is focused on the different isoforms of CD44. Ni teach that "CD44 (also known as Pgp-1, Hermes-3, HCAM, ECMR III) is a widely expressed glycoprotein with a molecular weight of 85 to 90 kDa."[38/] As discussed above, this passage cannot describe a CD44H or CD44R1 molecule because it is to small to possess the necessary carbohydrate modifications to possess E-selectin or L-selectin activity.

38/ Ni at page 1, first paragraph of Background Information.

The Examiner cites McEver for allegedly teaching that "the known manipulation and expression of interest that are associated with sialyated and fucosylated glycan and that interact with selectins."[39/] McEver teaches that the P-, E-, and L- selectins bind to sialylated, fucosylated, or sulfated glycans on glycoproteins. Specifically, McEver states as follows:

> The selectins bind sialyated and fucosylated oligosaccharides such as **sialyl Lewis x**..., a terminal component of glycans attached to glycoproteins.

This teaching is compared to the teaching in Figure 3 of Stamenkovic that demonstrates immunoprecipitation of the "hematopoietic form" of CD44 (CD44H) from CD44H transfected COS cells. The COS cell line is derived from kidney cells of the African Green monkey. As is explained in Sako[40/] COS cells are known to lack relevant fucosyltransferases essential for producing the sialofucosylated selectin binding determinants of the claimed glycosylated polypeptide. Specfically, Sako explains as follows:

> COS cells do not bind P-selectin **nor do they possess the appropriate glycosylation apparatus to synthesize Lewis" (Le") or SLe"**, presumed carbohydrate components of a P-selectin ligand.[41/]

CD44H-transfected COS cells thus cannot produce the claimed glycosylated polypeptide as COS cells natively lack the relevant fucosysltransferase to create HCELL.

Further, McEver actually teaches away from the present invention. The Specification teaches that CD44 glycoforms within the scope of the present claims bind to L-selectin in a sulfate independent manner. In contrast, McEver teaches that sulfation is required to bind L-selectin.[42/]

Finally, McEver in no way describes any isoforms (or glycoforms) of CD44.

2. A CD44 Glycoform Having E-Selectin or L-Selectin Ligand Activity is Not Taught in Any Reference Relied On By The Examiner

None of the primary references of Sackstein 1997, Stamenkovic, and Dougherty teach a CD44 glycoform having E-selectin or L-Selectin activity. Indeed, it was a general understanding

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39/ See Final Office Action at page 15.

40/ Sako *et al.*, "Expression cloning of a functional glycoprotein ligand for P-selectin," Cell. 1993 Dec 17;75(6):1179-86.

41/ Sako at page 1179, right column, 4th line of the Results section; citations omitted.

42/ See *e.g.*, McEver at page 586, right column, lines 8-9.

in the art that CD44 was not a selectin ligand. For example, Picker[43/] examined CD44 expressed on a human hematopoietic cell line and showed, specifically, that it lacked any HECA-452 reactivity by western blot. In another approach, Berg immunoprecipitated CD44 from a human "hematopoietic" (*i.e.*, hemopoietic) cell type (lymphocytes, obtained from human tonsil [lymph nodes]) and showed that isolated CD44H did not support binding of E-selectin-expressing cells ("ELAM-1-transfected cells"); however, protein(s) isolated by using HECA-452 mAb did support binding of E-selectin-expressing cells [44/] That is, isolated CD44 was used to show that the comparator protein (HECA-452 immunprecipitated protein(s) from the same tonsil [lymph node] lymphocytes) possessed E-selectin binding activity, but that CD44 did not possess such E-selectin binding activity. In this manner, CD44 was frequently used as a "negative control" specifically because it was shown to be non-reactive with HECA-452 mAb and found to be devoid of selectin ligand activity.[45/] This result applies to other CD44 isoforms isolated from other mammals. For example, Walcheck[46/] and Jutila[47/] each demonstrate that bovine CD44 is not involved in mediating bovine lymphocyte adhesion to E-selectin.[48/]

The sialylated, fucosylated glycan modifications confer a totally new biology to CD44 that was not appreciated, nor anticipated, in any of the references relied on by the Examiner. Prior to the present invention, it was thought that CD44 did not bind to any integral membrane protein(s), that CD44 only bound to extracellular matrix elements, and that no CD44-mediated adhesive interaction required divalent cations (which HCELL does to bind to either E- or L-selectin) . Further, as discussed above, whereas sialylation of CD44 is important for the selectin ligand activity of the presently claims glycoforms of CD44, sialylation of CD44 inhibits its

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43/ Picker *et al.*, "A Unique Phenotype of Skin-associated Lymphocytes in Humans", American Journal of Pathology, 136:1053-1068, 1990.

44/ See *e.g.*, Berg *et al.*, "The cutaneous lymphocyte antigen is a skin lymphocyte homing receptor for the vascular lectin endothelial cell-leukocyte adhesion molecule 1," J Exp Med. 1991;174:1461-1466.

45/ See *e.g.*, Berg *et al.*, "The cutaneous lymphocyte antigen is a skin lymphocyte homing receptor for the vascular lectin endothelial cell-leukocyte adhesion molecule 1," J Exp Med. 1991;174:1461-1466.

46/ Walcheck *et al.*, "Bovine gamma/delta T cells bind E-selectin via a novel glycoprotein receptor: first characterization of a lymphocyte/E-selectin interaction in an animal model," J Exp Med. 1993 Sep 1;178(3):853-63.

47/ Jutila *et al.*, "Cell surface P- and E-selectin support shear-dependent rolling of bovine gamma/delta T cells," J Immunol. 1994;153:3917-3928.

48/ See Walcheck at Abstract.

binding to hyaluronic acid.[49/] **On the basis of this physical chemistry and general understanding in the art, a person of ordinary skill in the art would not have considered the possibility that a glycoform of the "hyaluronic acid receptor" could be a selectin ligand.**

The CD44 glycoforms of the present claims were identified as E- and L-selectin ligands supporting binding E-selectin-expressing cells or lymphocytes (expressing L-selectin), respectively, under physiologically relevant shear-based assay conditions. To this end, the present inventor developed a novel and innovative technology, known as the "blot rolling assay". The blot rolling assay is a powerful technique, capable of discriminating and elucidating those membrane molecules that support adhesive interactions under appropriate shear conditions. A great advantage of this technique is that, within a complex mixture of membrane molecules, it allows identification of specific proteins that can mediate shear-resistant binding interactions under appropriate flow conditions, without requiring prior enrichment or isolation of such proteins.

In this assay, membranes are isolated from a cell type of interest, detergent solubilized, and the component proteins are resolved by gel electrophoresis and then transferred onto a support PVDF sheet (e.g., by western blot). The sheet is rendered translucent, then placed within a parallel plate flow chamber and mounted on an inverted microscope. Particles or cells bearing known adhesion molecules of interest (*e.g.*, Chinese hamster ovary cells stably transfected to express human E-selectin, or human lymphocytes expressing L-selectin) are then introduced into the chamber under controlled flow conditions, and the presence of tethering and rolling interactions on discrete bands can be observed by video microscopy. Pertinent substrate molecules supporting tethering/rolling of the flowing cells can thus be identified by immunostaining or by extraction of the band for mass spectrometry analysis or protein microsequencing.

Using this blot rolling assay it was demonstrated that the protein mediating certain adhesion events comprised a novel glycoform of CD44 containing HECA-452 reactive

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[49/] See *e.g.*, Katoh *et al.*, "Glycosylation of CD44 negatively regulates its recognition of hyaluronan;" J Exp Med. 1995 Aug 1;182(2):419-29. See also, Katoh *et al.*, "Cutting edge: an inducible sialidase regulates the hyaluronic acid binding ability of CD44-bearing human monocytes," J Immunol. 1999;162:5058-5061.

sialyated, fucosylated glycans, and that these glycans conferred E-selectin and L-Selectin ligand activity.[50/]

In view of the above, Applicant requests that this rejection be reversed.

3. Combining The References Would Not Have Lead A Person Of Ordinary Skill In The Art To The Claimed Purified Preparation

None of the primary references of Sackstein 1997, Stamenkovic, and Dougherty teach purified preparations of the recited CD44 glycoforms that comprises less than 5% of a polypeptide other than the glycosylated CD44 polypeptide according to the present claims.

Sackstein describes a polypeptide that was not MECA-79 reactive and therefore could **not** be immunoprecipitated from KG1a lysates using a MECA-79 antibody. The protein gels discussed in Sackstein 1997 are relied on by the Examiner are not purified preparations "containing less than 5% of a polypeptide other than the glycosylated CD44 polypeptide." Whole cell KG1a lysates were loaded onto the gels and these lysates would contain most, if not all, proteins from the KG1a cells. This is not a purified preparation containing less than 5% of any other polypeptide.

Stamenkovic described immunoprecipitation experiments in which whole cell lysates were placed on a gel. The protein gels discussed in Stamenkovic are relied on by the Examiner are not purified preparations "containing less than 5% of a polypeptide other than the glycosylated CD44 polypeptide." Whole cell COS cell lysates were loaded onto the gels and these lysates would contain most, if not all, proteins from the COS cells. This is not a purified preparation containing less than 5% of any other polypeptide.

The Western blot protein gels discussed in Dougherty relied on by the Examiner are not purified preparations "containing less than 5% of a polypeptide other than the glycosylated CD44 polypeptide." Again, however, whole cell KG1a lysates were loaded onto the gels and these lysates would contain most, if not all, proteins from the KG1a cells. This is not a purified preparation containing less than 5% of any other polypeptide.

[50/] See *e.g.*, Specification at Example 4.

More importantly, immunoprecipitation of CD44 from the cells as described in any of the references relied on by the Examiner could not produce a preparation of comprising less than 5% of a polypeptide other than the recited glycosylated CD44 polypeptide. In order to achieve this recited level of purity, a person of ordinary skill in the art would had to at least recognized the sialylated, fucosylated glycans as structural components of CD44 and then first immunoprecipitate CD44 and then immunoprecipitate with an antibody, such as HECA-452, that recognizes the relevant glycans. Accordingly, the cited references provides no guidance as to how to achieve the recited level of purity for the recited glycoform of CD44.

Further, the Examiner relies on Sackstein 1997, Stamenkovic, and Dougherty in the obviousness rejection without regard or appreciation of the CD44 glycoforms of the present claims. For example, on page 14, first paragraph of the Final Office Action the Examiner concludes that the CD44H and CD44R2 isoforms were previously described and that a person of ordinary skill in the art could "immediately envisaged" the CD44 glycoforms of the present claims. Specifically, the Examiner provides as follows:

> Again it is maintained that given the teachings of isolating and characterizing the structure of the claimed hemopoietic CD44H as well as CD44R1 and CD44R2, including their expression on various hemopoietic cells and cell lines, including the KGla cell lines. one of ordinary skill would have immediately envisaged or readily have expected the isolation of HCELL / KGla CD44 / CD44H / CD44R1 / CD44R2 glycosylated proteins including "isolated or purified protein that is substantially free of cellular materials or other contaminating protein from the cell or tissue source form which HCELL glycoprotein is derived or substantially freed form chemical precursors or other chemical when chemically synthesized , including being recombinantly produced, which is also free of culture medium (e.g. see page 11, paragraph 3 of the instant specification), thereby meeting the claimed limitation of "wherein the preparation comprises less that 5% of a polypeptide other than the glycosylated CD44 polypeptide" at the time the invention.[51/]

However, the Examiner provides no reasonable rationale as to why the references relied upon the Examiner would lead one of ordinary skill in the art to a CD44 molecules comprising sialylated, fucosylated glycans having E-selecin or L-selectin ligand activity. The Examiner simply concludes that because the CD44 polypeptides sequences were known, one of ordinary

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51/ Final Office Action at page 14, first full paragraph.

skill in the art could "immediately envisaged" the recited glycoforms of the polypeptides.[52/] Applicants submit that the above rationale is conclusory.

The secondary references Ni and McEver **cannot** cure the deficiencies of the primary references. Ni and McEver relied on by the Examiner to support the argument that one of ordinary skill in the art would have utilized the protein purification techniques disclosed in these references to isolate the ligand identified in Sackstein 1997, Stamenkovic, and Dougherty. On page 15 of the Final Office Action, the Examiner points to column 18, paragraph 1 of Ni for its teaching of "isolated" polypeptide. This passage, however, merely defines the term "isolated" as encompass[ing] remov[al] from its native environment, purified and produced by recombinant means" and that the term "isolated polypeptide" are "polypeptides that have been purified, partially or substantially, from a recombinant host cell."[53/] This passage merely indicates an understanding the art of the terms "isolated" and "purified". In any case, Ni refers to a polypeptide, not a distinguishing glycoform of a CD44 polypeptide as in the recited claims.

The Examiner cites McEver for its disclosure that "the known manipulation and expression of interest that are associated with sialyated and fucosylated glycan and that interact with selectins." See Office Action at page 13, fist paragraph. The Examiner specifically cites the columns 9-11 and 15-44, which provide guidance for the isolation and purification of recombinant proteins.

Further, Applicants submit the processes for the purification of proteins that would have been generally used by those of ordinary skill in the art (such as recombinant techniques) would not have led to the CD44 glycoproteins of the present claims as alleged by the Examiner. The production of a desired protein in a host cell generally comprises two basic steps: 1) identifying and isolating the gene encoding the desired protein; and 2) transferring the gene into the host cell. In general, the first step is by far the most difficult. Identifying the gene for a specific protein typically requires that at least a part of the nucleotide sequence of the gene be known. In most cases, this will involve inferring the nucleic acid sequence from the amino acid sequence of the protein. Although the techniques for amino acid sequencing are well known, obtaining a sample of the protein in sufficient quantity and purity for analysis can be quite difficult. Indeed,

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52/ See Final Office Action at page 14, second paragraph.

53/ See Final Office Action at page 15.

the decision to produce a protein by the methods of recombinant DNA technology is often motivated by the fact that such limited quantities of the protein are available from natural sources. The Examiner provides no reasonable rationale as to why a person of ordinary skill in the art, having identified the amino acid sequences of CD44 isoforms, would seek to obtain a purified preparation of a molecule not identified in the references cited by the Examiner. In any case, obtaining cDNA encoding CD44 would not disclose the invention. The references cited by the Examiner fail to appreciate that specific post-translational modifications would be critical to the structure of CD44 and that these glycan modifications would render the recited activities. The amino acid sequence of CD44 is essentially inert with regard to selectin ligand activity.

4. The Examiner has not made explicit an obviousness analysis that sets forth a reasonable rationale for maintaining this rejection

Applicant respectfully submit that the Examiner has not made explicit an obviousness analysis that sets forth a reasonable rationale for maintaining this rejection. The Examiner has failed to provide any rationale as to why a person of ordinary skill in the art would have been led to the invention now claimed. In contrast, Applicant has set forth above evidence supporting the conclusions that, prior to the present invention, a person of ordinary skill in the art would not motivated by the teachings of the cited references to purify a preparation comprising a CD44 glycoform modified with sialylated, fucosylated glycans and having E-selectin or L-selectin binding activity. The following three points support this conclusion:

1. Maiti[54/] was offered as evidence that a person of ordinary skill in the art would have considered that sulfation was required for CD44-mediated leukocyte adhesion; in contrast, Sackstein 1997 expressly teaches that the identified L-selectin binding activity was sulfate independent.[55/]

2. Stamenkovic teaches that the hematopoietic form of CD44, or CD44H, recognizes hyaluronate (HA) and that "this recognition appears to account for all the

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54/ Maiti A, Maki G, Johnson P.; TNF-alpha induction of CD44-mediated leukocyte adhesion by sulfation.

Science. 1998 Oct 30;282(5390):941-3.

55/ Sackstein 1997 at Abstract.

previously proposed adhesion specificities."[56/] Indeed, CD44 was generally referred to in the art as the "hyaluronic acid (HA) receptor". In contrast, Katoh[57/] showed that this adhesive function of CD44 is actually inhibited by certain carbohydrate modifications, such as sialic acid. Thus, a CD44 glycoform comprising sialylated, fucosylated glycans would have not been thought to possess any adhesion specificity, let alone identified as an E- or L- selectin ligand.

3. Indeed, CD44 was known to lack HECA-452 reactivity, and was often used as a "negative control" for E-selectin (ELAM-1) binding, as in Berg.[58/] The teachings of Picker[59/], Berg[60/], Jutila[61/], and Walcheck[62/] each, and altogether, provide evidence for the well-established (prior) canon in the art — that is, it was generally understood in the art at the time of invention that CD44 was not a selectin ligand.

In contrast, the Examiner merely relies on a hindsight rationale and conclusory statements that a person of ordinary skill in the art would have immediately envisioned the claimed preparations. This is improper. In every possible way, all the prior information of CD44 structural biology and function would have taught away one of skill in the art from perceiving that CD44 could serve as a selectin ligand. Accordingly, the Examiner has failed to set forth a *prima facie* case of obviousness.

56/ Stamenkovic at page 343, right column, lines 3-7.

57/ Katoh S, Zheng Z, Oritani K, Shimozato T, Kincade PW.; "Glycosylation of CD44 negatively regulates its recognition of hyaluronan;" J Exp Med. 1995 Aug 1;182(2):419-29.

58/ See *e.g.*, Berg *et al.*, "The cutaneous lymphocyte antigen is a skin lymphocyte homing receptor for the vascular lectin endothelial cell-leukocyte adhesion molecule 1," J Exp Med. 1991;174:1461-1466.

59/ Picker *et al.*, "A Unique Phenotype of Skin-associated Lymphocytes in Humans", American Journal of Pathology, 136:1053-1068, 1990.

60/ Berg *et al.*, "The cutaneous lymphocyte antigen is a skin lymphocyte homing receptor for the vascular lectin endothelial cell-leukocyte adhesion molecule 1," J Exp Med. 1991;174:1461-1466.

61/ Jutila *et al.*, "Cell surface P- and E-selectin support shear-dependent rolling of bovine gamma/delta T cells," J Immunol. 1994;153:3917-3928

62/ Walcheck *et al.*, "Bovine gamma/delta T cells bind E-selectin via a novel glycoprotein receptor: first characterization of a lymphocyte/E-selectin interaction in an animal model," J Exp Med. 1993 Sep 1;178(3):853-63.

CONCLUSION

An indication of allowance of all claims is respectfully solicited. Early notification of a favorable consideration is respectfully requested. In the event any issues remain, Applicants would appreciate the courtesy of a telephone call to their counsel to resolve such issues and place all claims in condition for allowance.

Respectfully submitted,

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